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INTL ISOCYANATE INST., INC.

Contractor
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Document Title
LITERATURE STUDY ON REACTION OF ISOCYANATES WITH BIOLOGICAL MATERIALS WITH ATTACHMENTS AND COVER LETTER DATED 072287

Chemical Category
TOLUENE DIISOCYANATE (1321-38-6)

INTERNATIONAL ISOCYANATE INSTITUTE, INC.
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22 July 1987

86-870000612

Document Processing Center (TS-790)
Office of Toxic Substances
Environmental Protection Agency
401 M Street, S.W.
Washington, D. C. 20460

Attention: 8(d) HEALTH and SAFETY REPORTING RULE (REPORTING)
May 1, 1987

Dear Sir or Madam:

As described at 40 C.F.R. 716.20(a) (10), the International Isocyanate Institute (III) submits the enclosed studies on behalf of its members to satisfy member reporting requirements under Section 8(d) of the Toxic Substances Control Act. These studies are on chemicals added to the 8(d) list on May 1, 1987. The studies are indexed by CAS numbers with chemical name, III identification number and title provided.

Attachment #1 is an indexed list of completed studies.

Attachment #2 is a compilation of the reports from the completed studies.

Attachment #3 is an indexed list of studies that are currently in progress.

Please refer to the III identification number in any communication regarding the report.

If the Agency needs further information, please do not hesitate to contact me.

Very truly yours,

R. K. Rigger
Managing Director

RKR/c
enclosures

86-870000612

ATTACHMENT #1

INDEXED LIST OF COMPLETED STUDIES

CAS # 101-68-8 Benzene, 1,1'-methylenebis[4-isocyanato-
Methylenedi-p-phenylene diisocyanate
4,4'-Methylenebis(phenyl isocyanate)
MDI
4,4'-Diisocyanatodiphenylmethane

III NUMBER	TITLE
10000	Prepolymeric MDI (Biphenylmethane Diisocyanat) with and without added Phenyl Isocyanate (PhI) - one hour acute inhalation toxicity.
10005	Determination of the concentration of vapor generated from monomeric 4.4'-Diphenylmethane Diisocyanate (MDI) by a dynamic method.
10008	Two-day study into the relation between polymeric MDI concentration values obtained by a QCM-Cascade, HPLC and Colorimetry.
10010	Liquid Waste after TDI/MDI decontamination.
10012	Literature Study on Reaction of Isocyanates with Biological Materials.
10013	Report on fire hazard of Isocyanate chemicals.
10014	Report on fire hazard of Isocyanate chemicals.
10018	Analytical methods to monitor aerosols of Polymeric 4,4'-Diphenylmethane-diisocyanate (MDI) at low concentrations.
10019	Aquatic life study phase II, step 2 Accumulation of TDI, MDI, TDA and MDA in fish and their toxicity.
10022	Generation and monitoring of breathable aerosols of polymeric 4,4'-diphenylmethane-diisocyanate (MDI).

ATTACHMENT #1

INDEXED LIST OF COMPLETED STUDIES

CAS #101-68-8 Benzene, 1,1'-methylenebis[4-isocyanato-
Methylenedi-p-phenylene diisocyanate
4,4'-Methylenebis(phenyl isocyanate)
MDI
4,4'-Diisocyanatodiphenylmethane

III NUMBER	TITLE
10026	Pre-polymeric diphenylmethane,4,4', diisocyanate (Petmar MDI) Pre-polymeric diphenylmethane,4,4', diisocyanate + phenyl isocyanate. 50 ppm. Pre-polymeric diphenylmethane,4,4', diisocyanate + phenyl isocyanate. 150 ppm. An experiment to investigate the relative sub-acute toxicity of the above substances in the rat by inhalation.
10050	Metabolism and toxicogenetics of Methylenedianiline.
10065	A study of the diffusion of MDI in rats contaminated via the respiratory system.
10074	Investigations on the microbial degradation of PU forams. Part II.
10075	Respiratory Sensitivity Study.
10076	Deposition of aerosol components on the hair of rats exposed to polymeric MDI aerosols.
10077	Acute inhalation toxicity study of polymeric MDI in rats.
10092	Biological action of TDI and MDI in water.
10129	Immunological aspects of Isocyanates.
10187	Isocyanates : Irritation and Hypersensitivity.
10188	Preliminary study on skin sensitization caused by MDI solutions.

ATTACHMENT #1

INDEXED LIST OF COMPLETED STUDIES

CAS # 101-68-8 Benzene, 1,1'-methylenebis[4-isocyanato-
Methylenedi-p-phenylene diisocyanate
4,4'-Methylenebis(phenyl isocyanate)
MDI
4,4'-Diisocyanatodiphenylmethane

III NUMBER	TITLE
10206	Aquatic life study Phase II, Step 2, Accumulation of TDI, MDI and their reaction products in Daphnia.
10223	TDI and MDI immunological studies. Summary report of research supported by the International Isocyanate Institute.
10234	Aquatic life study Phase II, Step 1. Biodegradation of TDI and MDI in the model river and marine water.
10243	Mortality among workers exposed to isocyanates. Feasibility Study.
10253	Sub-chronic (13 week) inhalation toxicity study of polymeric MDI aerosol in rats (part B2)
10258	Ecotoxicity of Toluenediisocyanate (TDI) Diphenylmethanediisocyanate (MDI) Toluenediamine (TDA) Diphenylmethanediamine (MDA)
10299	Aquatic Life Studies
10317	Production and control of breathable MDI aerosols for primal experiments.

ATTACHMENT #1

INDEXED LIST OF COMPLETED STUDIES

CAS # 101-68-8 Benzene, 1,1'-methylenebis[4-isocyanato-
Methylenedi-p-phenylene diisocyanate
4,4'-Methylenebis(phenyl isocyanate)
MDI
4,4'-Diisocyanatodiphenylmethane

IUI NUMBER	TITLE
10360	Generation of 4,4' Diphenylmethane Diisocyanate (MDI) vapour
10386	Pharmacokinetics of MDI after inhalation exposure of rats to labelled MDI.
10391	Skin sensitization by isocyanates.
10393	Study of the burning characteristics of isocyanate chemicals.
10439	Di-Isocyanate Induced Asthma - Reactions to TDI, MDI, HDI and Hisamine.
24298	Acute Inhalation Toxicity (LC_{50}) in the Male Albino Rat.

ATTACHMENT #1

INDEXED LIST OF COMPLETED STUDIES

CAS #1321-38-6 Benzene, diisocyanatomethyl-(unspecified isomer)

III NUMBER	TITLE
10010	Liquid waste after TDI/MDI decontamination.
10012	Literature Study on Reaction of Isocyanates with Biological Materials.
10013	Report on fire hazard of Isocyanate chemicals.
10014	Report on fire hazard of Isocyanate chemicals.
10019	Aquatic life study phase II, step 2 Accumulation of TDI, MDI, TDA and MDA in fish and their toxicity.
10024	Tolylene di-isocyanate three week inhalation toxicity in the rat.
10033	Stack Emission Part B : Emitted TDI Gas Treatment with Activated Carbon.
10034	Stack Emission Part A : Emitted TDI Gas Treatment with Activated Sludge.
10035	The toxicity and carcinogenicity to rats of Toluene Diisocyanate vapour administered by inhalation for a period of 113 weeks.
10040	Reaction of TDI with water and with wet sand.
10044	Emission of Tolylene Diisocyanate (TDI) and Tolylene Diamine (TDA) in flexible polyurethane foam production lines.
10045	Emission of Tolylene Diisocyanate (TDI) and amines.
10055	Preparation and evaluation of a system for exposing rats to Toluene Diisocyanate vapour.

ATTACHMENT #1

INDEXED LIST OF COMPLETED STUDIES

CAS # 1321-38-6 Benzene, diisocyanatomethyl- (unspecified isomer)

III NUMBER	TITLE
10057	Evaluation of a system for exposing hamsters to Toluene Diisocyanate vapour.
10064	A study of the diffusion rate of TDI in rats contaminated via the respiratory system.
10074	Investigations on the microbial degradation of PU foams. Part II
10075	Respiratory sensitivity study.
10089	Studies of Toluene Diisocyanate induced pulmonary disease.
10092	Biological action of TDI and MDI in water.
10094	Foam plant stack emission data.
10095	Stack Emission Part B : Emitted TDI Gas Treatment with Activated Carbon "Regeneration of Spent Activated Carbon".
10096	Stack Emission Part A : Emitted TDI Gas Treatment with Activated Sludge.
10098	Epidemiological study for effects of TDI.
10100	Histopathological observations on selected tissues of syrian hamsters exposed by inhalation to vapors of Toluene Diisocyanate (TDI) for 6 hours/day, 5 days/week for 4 weeks.
10116	Review of the incidence of rhinitis in rats exposed chronically to Toluene Diisocyanate vapour.

ATTACHMENT #1

INDEXED LIST OF COMPLETED STUDIES

CAS # 1321-38-6 Benzene, diisocyanatomethyl- (unspecified isomer)

III NUMBER	TITLE
10117	Review of the national toxicology program carcinogenesis bioassay of Toluene Diisocyanate.
10121	Toluene Diisocyanate (TDI) proposed exposure standard.
10129	Immunological aspects of Isocyanates.
10142	Toluene Diisocyanate acute inhalation toxicity in the rat.
10153	A 30-day repeated inhalation toxicity study of Toluene Diisocyanate (TDI) in laboratory animals.
10159	The fate of Toluene Diisocyanate.
10162	Epidemiological study for effects of TDI.
10163	Validation of MCM 4000 personal monitor and MCM 4100 integrating reader/recorder system.
10168	Summary of work carried out on FE-A-14 III - 1 by H. Sakurai and co-workers.
10169	The toxi ity and carcinogenicity to rats of Toluene Diisocyanate vapour administered by inhalation for a period of 113 weeks.
10175	Emission of Tolylene Diisocyanate (TDI) and Tolylene Diamine (TDA) in flexible polyure.ane foam production lines.
10184	Immunological studies on TDI exposed workers. Part I.
10187	Isocyanates . Irritation and Hypersensitivity.

ATTACHMENT #1

INDEXED LIST OF COMPLETED STUDIES

CAS # 1321-38-6 Benzene, diisocyanatomethyl- (unspecified isomer)

III NUMBER	TITLE
10206	Aquatic life study Phase II, Step , Accumulation of TDI, MDI and their reaction products in Daphnia.
10208	The Toxicity and Carcinogenicity to rats of Toluene Diisocyanate vapour administered by inhalation for a period of 113 weeks. Addendum Report. Vol. 2.
10210	The Toxicity and Carcinogenicity to rats of Toluene Diisocyanate vapour administered by inhalation for a period of 113 weeks. Vol. I
10223	TDI and MDI immunological studies. Summary report of research supported by the International Isocyanate Institute.
10233	The Toxicity and Carcinogenicity to rats of Toluene Diisocyanate vapour administered by inhalation for a period of 113 weeks. Addendum Report. Vol. 1
10234	Aquatic life study Phase II, Step 1. Biodegradation of TDI and MDI in the model river and marine water.
10237	Isocyanate monomer in PU foam.
10243	Mortality among workers exposed to isocyanates. Feasibility Study.
10258	Ecotoxicity of Toluenediisocyanate (TDI). Diphenylmethanediisocyanate (MDI) Toluenediamine (TDA). Diphenylmethanediamine (MDA)
10259	Sampling and Analysis of TDI atmospheres at Klinikum Grosshadern, Munich.

ATTACHMENT #1

INDEXED LIST OF COMPLETED STUDIES

CAS # 1321-38-6 Benzene, diisocyanatomethyl- (unspecified isomer)

III NUMBER	TITLE
10299	Aquatic Life Studies.
10307	Studies on the effects of TDI on living animals.
10308	Change of TDI in olive oil.
10321	Improvement in RAST for TDI. Parts A and B.
10340	Audit of the national toxicology program carcinogenesis bioassay of toluene diisocyanate.
10345	Isocyanate spillage control.
10348	Immunological Studies on TDI exposed workers Part II.
10349	Isocyanate hypersensitivity.
10382	The toxicity and carcinogenicity of Toluene Diisocyanate vapour when administered to mice over a period of approximately 2 years. Summary Report.
10383	The toxicity and carcinogenicity of Toluene Diisocyanate vapour when administered to mice over a period of approximately 2 years.
10391	Skin sensitization by isocyanates.
10393	Study of the burning characteristics of isocyanate chemicals.

ATTACHMENT #1

INDEXED LIST OF COMPLETED STUDIES

CAS #1321-38-6 Benzene, diisocyanatomethyl-(unspecified isomer)

III NUMBER	TITLE
10416	Sampling and analysis of TDI atmospheres at Klinikum Grosshadern, Munich.
10430	Protective effect of drugs on late asthmatic reactions and increased airway responsiveness induced by Toluene Diisocyanate in sensitized subjects.
10433	The reactions of OH radicals with Toluene Diisocyanate, Toluenediamine, and Methylene Dianiline under simulated atmospheric conditions.
10434	Metabolism and disposition of ^{14}C-labeled Toluene Diisocyanate (TDI) following oral and inhalation exposure ; Preliminary studies.
10437	Toluene Diisocyanate-Induced Asthma: Bronchial Provocation and Reactivity Studies.
10438	Toluene Diisocyanate-Induced Asthma: Inhalation Challenge Tests and Bronchial Reactivity Studies.
10439	Di-Isocyanate Induced Asthma- Reactions to TDI, MDI, HDI and Histamine.

ATTACHMENT #1

INDEXED LIST OF COMPLETED STUDIES

CAS # 91-08-07 Benzene, 1,3-diisocyanato-2-methyl
TDI, 2-6-diisocyanate

TII NUMBER	TITLE
24207	Disposition of 2,6-Toluene Diisocyanate in Fischer 344 rats

ATTACHMENT #2

COMPILATION OF REPORTS FROM III FILES
(AS INDEXED IN ATTACHMENT #1)

These reports are in envelopes labeled Attachment #2 and are packaged, along with an envelope, addressed to:

Document Processing Center (TS-790)
Office of Toxic Substances
Environmental Protection Agency
401 M Street, S.W.
Washington, D. C. 20460

Attention: 8(d) HEALTH and SAFETY REPORTING RULE
(REPORTING) May 1, 1987

from:

International Isocyanate Institute, Inc.
119 Cherry Hill Road
Parsippany, New Jersey 07054

containing a transmittal letter for these documents.

ATTACHMENT #3

INDEXED LIST OF STUDIES IN PROGRESS

CAS # 101-68-8 Benzene, 1,1'-methylenebis[4-isocyanato-
Methylenedi-p-phenylene diisocyanate
4,4'-Methylenebis (phenyl isocyanate)
MDI
4,4'-Diisocyanatodiphenylmethane

III NUMBER	TITLE
E-A-8	Study of chronic toxicity and carcinogenicity of polymeric MDI aerosol in rats. Part C Study. Current work authorized to begin June 1985. To study chronic toxicity and carcinogenicity of polymeric MDI aerosol in rats. Data sought - Effect on animal tissues. Our current estimated completion date for this study is the first quarter of 1989. It may be possible to complete this study before 1989; however, it may require more time. CIVO Institution, Tno., Toxicology and Nutrition, Utrechtsewe 848, P.O. Box 306, 3700 A.J. Zeist, The Netherlands.
E-H-44	MDI sampling and analysis at CIVO Current work authorized to begin November 1984. To study consistency/comparability of various methods continuous/discontinuous for determining the composition of atmospheres in Study E-A-8 (Part C) above. Data sought - Analytical data on polymeric MDI aerosol atmospheres. Our current estimated completion date for this study is the first quarter 1989. It may be possible to complete this study before 1989; however, it may require more time. CIVO Institution, Tno., Toxicology and Nutrition, Utrechtsewe 848, P.O. Box 306, 3700 A.J. Zeist, The Netherlands.

ATTACHMENT #3

INDEXED LIST OF STUDIES IN PROGRESS

CAS # 1321-38-6 Benzene, diisocyanatomethyl- (unspecified isomer)

III NUMBER	TITLE
E-B-11	Epidemiological study of workers in U.K. flexible foam industries.

Current work authorized to begin Mid 1978.
To investigate whether working on flexible PU foam manufacturing plants gives rise to increased expectation of decrements in lung parameters above those due to ageing.
Data sought - monitoring of exposed workers' and controls' lung parameters. Monitoring of airborne TDI (and on limited scale of tertiary aliphatic amine) in the workplace.
Our current estimated completion date for this study is the first quarter of 1989. It may be possible to complete this study before 1989; however, it may require more time.
Tynestead Limited, Tynestead House, 22 Camberley Drive, Bamford, Rochdale, Lancs, OL11 4 AZ, UK. and Medical Research Council, 20 Park Crescent, London, UK.

ATTACHMENT # 3

INDEXED LIST OF STUDIES IN PROGRESS

CAS # 1321-38-6 Benzene, diisocyanatomethyl- (unspecified isomer)

III NUMBER	TITLE
FE-AB-14	Epidemiological study of workers in Japan flexible foam industries. Phase V.

Current work authorized to begin August 1985.
To clarify relationship between TDI concentration and chronological change in pulmonary and respiratory symptoms of workers in PU foam plants. Data sought.
Monitoring of exposed workers' and controls' lung parameters.
Monitoring of airborne TDI in the workplace.
Our current estimated completion date for this study is the first quarter of 1989. It may be possible to complete this study before 1989; however, it may require more time.
School of Medicine, Keio University, Shinjuki-Ku, Tokyo, Japan.

ATTACHMENT # 3

INDEXED LIST OF STUDIES IN PROGRESS

CAS # 1321-38-6 Benzene, diisocyanatomethyl- (unspecified isomer)

III NUMBER	TITLE
E-E-22	Clean Stack Air Project Current work authorized to begin March 1980. To study ways in which TDI Emissions from flexible foam plants can be removed from exhaust gases by carbon absorption. Data sought - Concentrations of TDI at inlets and outlets of carbon absorption units. Our current estimated completion date for this study is the first quarter of 1989. It may be possible to complete this study before 1989; however, it may require more time. Dunlop (Now BTR, Silvertown House, Vincent Square, London, UK.
E-AB-40	An investigation into the mortality and cancer morbidity of production workers in the UK flexible polyurethane foam industry. Current work authorized to begin July 1987. To compare the mortality and cancer morbidity experience of production workers in UK flexible foam manufacturing plants with those of unexposed controls and of the population at large, and to determine, if appropriate, possible reasons for differing experiences. Data sought. Comparative Data on death and illness due to cancer, analysed statistically. Data sought. The expected date of termination of project is indeterminate since it depends on results found at different intervals. The first analysis will take place 1989. Cancer Epidemiology Unit, University of Birmingham, Edgbaston, Birmingham UK.

ATTACHMENT #3

INDEXED LIST OF STUDIES IN PROGRESS

CAS #1321-38-6 Benzene, diisocyanatomethyl- (unspecified isomer)

III NUMBER	TITLE
NA-E-24	Fate of airborn TDI (Part II)

Current work authorized to begin May 1984.
To determine the fate of airborne TDI and the effects of moisture, light, and atmospheric pollutants on TDI loss from the gas phase.
Our current estimated completion date for this study is the first quarter of 1989. It may be possible to complete this study before 1989; however, it may require more time.
Battelle Columbus Laboratories, 505 King Avenue, Columbus, Ohio 43201

NA-AB-26 Detecting delayed isocyanate sensitivity.

Current work authorized to begin May 1, 1987.
This research is being conducted to better detect delayed isocyanate sensitivity in persons exposed and/or sensitized to isocyanates. In 1986, M. Karol's work was directed towards identification of isocyanate-specific lymphocytes by class.
Our current estimated completion date for this study is the first quarter of 1989. It may be possible to complete this study before 1989; however, it may require more time.
Dr. M. Karol, University of Pittsburgh, 130 Desoto Street, Pittsburgh, Pennsylvania 15261

ATTACHMENT #3

INDEXED LIST OF STUDIES IN PROGRESS

CAS #1321-38-6 Benzene, diisocyanatomethyl- (unspecified isomer)

III NUMBER	TITLE
NA-AB-43	Improvement of RAST tests for TDI

Current work authorized to begin May 1, 1987.
This research is being conducted to improve RAST (Radiolabeled Antibody Sorbent Technique) test for identifying exposure and sensitization to TDI. Additional mechanistic work on TDI sensitization is being conducted by Dr Brown. This includes studying proteins in TDI exposed animals.
Our current estimated completion date for this study is the first quarter of 1989. It may be possible to complete this study before 1989; however, it may require more time.
Dr W. E. Brown, Carnegie-Mellon University, Pittsburgh, Pa. 15261.

NA-AB-50 TDI Reprotoxicity

The teratology study was initiated in the 4th quarter of 1986.
The reproduction study was initiated in the 2nd quarter of 1987.
This project evaluates both the "Developmental Toxicity of Inhaled TDI in CD (Sprague-Dawley) Rats" and "Two-Generation Reproduction Toxicity of TDI in CD (Sprague-Dawley) Rats."
Our current estimated completion date for this study is the first quarter of 1989. It may be possible to complete this study before 1989; however, it may require more time.
Dr T. W. Tyl, Bushy Run Research Center, RD #4, Mellon Road, Export, Pennsylvania 15632.

10012.

LITERATURE STUDY

ON REACTION OF ISOCYANATES

WITH BIOLOGICAL MATERIALS

The International Isocyanate
Institute, Inc.
71 Elm Street
New Canaan, CT 06840 / USA

Project No. EB 23

Performed by

Dr. F.K. Brochhagen
Bayer AG
PU-A/S
D-5090 Leverkusen, Bayerwerk

and

Dr. D. Dieterich
Bayer AG
PU-F 1
D-5090 Leverkusen, Bayerwerk

August 11, 1982

S U M M A R Y

Literature Study on Reaction of Isocyanates with Biological Materials

Introduction

It is known that isocyanates - particularly toluenediisocyanate (TDI) and diphenylmethanediisocyanate (MDI) - if inhaled or otherwise administered can cause injuries to the health of humans. As a consequence of this exposure an interaction with reactive groups of human proteins has to be expected due to the high reactivity of these chemicals. There is an ongoing discussion in the scientific world whether the effects of isocyanates on the health of humans follow immunological, pharmacological or other mechanisms in which adducts of isocyanates and proteins might be involved.

In order to evaluate the literature related to this topic the International Isocyanate Institute, Inc. has contracted the aforementioned study to Bayer AG by letter of July 8, 1981. The respective literature references which appeared in Chemical Abstracts from 1972 through 1981 have been considered. In addition several earlier publications were studied also. The references on some literature which is not directly related to the objectives of the study have been included (3.7, 3.15, 3.16, 3.18, 6.1 and 6.2).

It was deemed appropriate to classify the outcome of the study into the following headings:

1. Basic studies on the reaction of proteins with mono- and diisocyanates;

2. Interaction between isocyanates and enzymes;

3. Antigens based on adducts of isocyanates and proteins;

4. Pharmacological effects of isocyanates;

5. Nitrosoureas - Supposed pharmacological mechanism of antitumor activity;

6. Isocyanates acting as antagonist for carcinogens.

Specific comments

To 1 (6 references):

Reactions with monoisocyanates have been performed in order to identify reactive sites in aminoacids and peptides; diisocyanates have been used as coupling agents, e.g. to combine a biological active protein with a "label" compound. Reactions of this type are of interest in view of the synthesis of antigens by reaction of isocyanates and HSA. Free NH_2- and OH-groups of the proteins were the preferred reaction site for the isocyanates.

In most cases the reactions have been carried out in an aqueous environment. By-products of such coupling procedures may therefore be formed in considerable amounts. This possibility has been disregarded more or less; only limited work has been done to identify by-products.

To 2 (18 references):

W.E. Brown, F. Wold and al, University of Minnesota, have performed a series of basic studies (1971 - 1975) on the influence of aliphatic isocyanates (mainly butyl- and octyl-) on the activity of various enzymes. Inhibition of the enzyme's activity has been shown in most cases; the proteinic -SH- group is the preferred reaction site for the isocyanate. The length of its aliphatic chain and the size of the protein's "binding pocket" are correlated to each other. Side reactions of the isocyanate in the aqueous environment are taken into account to some extent.

Several other authors basing on the work of the Brown/Wold group have also studied the influence of aliphatic isocyanates (including chloroethyl- and chloromethyl-) on the activity of enzymes. Effects similar to the Brown/Wold findings were observed i.e. inactivation of the enzyme in most cases. Isocyanate derivates of penicillin have an inhibitory effect on lactamase. Two recent papers describe the inhibition of cholinesterase by various isocyanates (including TDI) in vitro. This of course has also pharmacological implications.

To 3 (21 references):

1964 Scheel postulated an immunologic mechanism via the in vivo reaction of TDI with protein thus forming an antigen which prompts formation of IgE antibodies.

In order to detect these antibodies, e.g. in a radioimmuno-assay TDI was reacted in vitro with e.g. human serum albumin (HSA) to form a synthetic antigen with a tolyl hapten group.

Some authors confirmed this by finding a relationship between the occurrence of such antibodies and symptoms of diisocyanate hypersensitivity, but other authors found contradictory results.

1978 Karol et al. used p-tolyl-mono-isocyanate instead of TDI for antigen preparation in vitro, thereby avoiding crosslinking reactions and ensuring the formation of sterically exposed tolyl hapten groups. This artificial test-antigen proved rather successful in detecting TDI antibodies. This is not confirmed by other authors.

To 4 (6 references):

Since before 1978 there was no unequivocal evidence of antibodies caused by TDI exposure, some authors were looking for a direct pharmacological effect of TDI. Special attention has been given to the action of TDI on lymphocyte cyclic adenosin monophosphate (cAMP) in connection with investigation of the beta-adrenergic function.

To 5 (6 references):

Basing on the observation that nitroso-(chloro)alkyl-ureas are effective antitumor agents several authors establish the hypothesis that the isocyanates (chloroethyl- and cyclohexyl-) which were used to synthetize the ureas will be formed in vitro from the ureas. Basing on this assumption they claim (and confirm to some extent by in vitro studies) that the antitumor activity of the ureas is caused by the respective isocyanates. The authors do not take into account that from the chemical point of view the generation of isocyanates from ureas under the conditions of the described tests must generally be excluded.

To 6 (2 references):

Various isocyanates - especially aryl-derivates - were applied to rodents which had been treated with tumor cells or a carcinogenic chemical. They inhibited the growth of tumor cells respectively the occurrence of neoplasia.

Conclusions and recommendations

The literature study leads to the following conclusions:

- Many approaches have been made to evaluate the biological effects of isocyanates in vitro and in vivo; a systematic manner, however, to perform the studies is missing in most cases.

- Remarkable biological phenomena were observed in several cases but the limited knowledge on the reaction behavior of isocyanates in an aqueous biological environment often led to false interpretations.

- The isocyanates react obviously with reactive sites of the protein; in general a denaturation of the protein does not occur.

- There is little doubt that health injuries which might occur by isocyanates exposure involve an immunological mechanism; it seems, however, that a contribution of pharmacological effects to the clinical phenomena cannot be excluded.

It is recommended:

- In order to increase the basic knowledge on the reaction of mono- and diisocyanates with proteins, <u>further in vitro studies preferably with proteins bearing hydrophobic areas should be performed in an aqueous biological environment.</u>

- Subsequently in vitro studies on the reaction of TDI and MDI with biological materials under biological conditions should be initiated. The aim of the study should be to identify the adducts by appropriate methods and to investigate their immunochemical and pharmacological behavior.

- Further I.1.I. funded studies on the direct immunological and pharmacological effects of diisocyanates on humans and test animals should be postponed until the behavior of possible isocyanate/protein adducts has been clarified.

1. Basic studies on the reaction of proteins with mono- and diisocyanates

1.1 H. Fraenkel-Conrat, M. Cooper, H.S. Olcott: Action of Aromatic Isocyanates on Proteins

Jour. Amer. Chem. Soc. 1945, 67, 314-319

Proteins (e.g. egg albumin, gliadin, casein) as well as several amino-acids were treated with phenylisocyanate (and several other aromatic isocyanates) under anhydrous conditions in dry pyridine at 70°C for 24-96 hours. Reaction occurred with the basic groups (amino, guanidyl, imidazole); the acid groups (carboxyl, phenolic) and the amide groups. There was no appreciable reaction under these conditions with the peptide groups of chain molecules. The reaction between egg white protein and phenylisocyanate in aqueous conditions was studied for comparison. Little reaction of acid groups of proteins were found in an acid medium, none in alkaline solution.

The rate of reaction was assessed by determining the amount of non reacted active groups.

1.2 A.F. Schick, S.J. Singer: On the Formation of Covalent Linkages between two Protein Molecules

J. Biol. Chem. 236, 2477-2485 (1961)

This is a detailed study how to link two protein molecules together in a binary soluble conjugate by stable covalent bonds while retaining the biological activity of at least one of them (the antibody). Conjugates of bovine serum albumin with bovine γ-globulin, and of ferritin with rabbit γ-globulin (containing antibody) were prepared by the use of a number of diisocyanates, e.g. xylylene diisocyanate, 3-methoxy diphenylmethane-4,4'-diisocyanate and 2,4'-toluene diisocyanate. The reactions were performed in an aqueous environment (borate buffer, pH 9,5 and phosphate buffer, pH 7,5). With 2,4-TDI, conditions were found for producing exclusively covalently-linked conjugates in good yield. The presence of conjugates was determined electrophoretically. NH_2-groups of the proteins were confirmed to be the active site as reaction partner for the NCO-group. The reaction mechanisms of diisocyanates bearing NCO-groups of different activities as well as the differences of reactivity of active groups of the proteins have been taken into account by the authors.

1.3 H. Fasold,
F. Turba:

Spaltbare Peptidbrücken-bildende Di-Isocyanate von Azoverbindungen

Biochemische Zeitschrift 337, 80-87 (1963)

Azophenyl-p-diisocyanate has been found to be a reagent for linking two functional groups in in peptide chains. The reaction of this diisocyanate with leucine and lysin derivatives is described. A water/dioxane mixture was used as solvent. Besides of the diureido compounds formed from the amino-acids and the diisocyanate monoureido compounds have been identified which are formed by partial hydrolysis of the isocyanate.

1.4 H. Fasold:

Zur chemischen Untersuchung der Tertiärstruktur von Proteinen

Biochemische Zeitschrift 342, 288-294 (1965)

2,2'-dicarboxy-4,4'-azophenyldiisocyanate is preferred to azophenyl-p-diisocyanate as cross-linker for proteins due to its much better solubility in water. The author describes the formation of an azoprotein from this carbyxylated diisocyanate and sperm whale myoglobin. The reaction is performed in a buffered aqueous solution of the protein; the diisocyanate is added in dimethylformamide (DMF) solution. DMF was only used in very small amounts which did not cause a denaturation of the protein. Reaction products of the diisocyanate with water were separated by ultracentrifuge treatment. The remaining solution which did contain the "azomyoglybin" was compared with a myoglobin solution which had the same molar concentration of the protein. Treatment of these solutions with bromoacetic acid which leads to a carboxymethylation of the histidine part of the protein chain has confirmed that the isocyanate treated in myoglobin does still have its native structure.

1.5 H. Ozawa:

Bridging Reagent for Protein

Biochemistry, vol. 62, no. 4, 419-423, 1967

The adduct of 1 mol hexamethylene diisocyanate and 2 moles of l-lysine was prepared by combining HDI in acetone solution with the amino acid-hydrochloride in form of its copper complex. The pure HDI-lysine (with the HDI linked to the ϵ-amino groups) was isolated in crystalline form. The product is hydrolysed partially by treatment with hydrochloric acid at 110°C.

HDI was also combined with ribonuclease (R-nase) at the same condtions as with lysine. Depending on the fraction of the adduct (HDI-R-nases 1, 2, 3, 4) the enzymatic activity is not changed (HDI-R-nase 1) or the enzyme becomes completely inactive (HDI-R-nases 3 and 4). It is shown that HDI reacts with the ϵ-amino group of the lysine component of R-nase. Other amino acids in the enzyme were not derivatised by the isocyanate. In case of HDI-R-nase 1 it has been shown that 1 mol HDI has reacted with 1.8 moles of lysine which suggest that HDI acts as a bridging agent between 2 R-nase molecules.

Studies on the reaction between chymotrypsin and hexamethylene diisocyanate showed a complete inactivation of the enzyme in a very short time. It is assumed that HDI and water/acetone reacts with the serine part of chymotrypsin.

1.6 C. J. Sanderson:

Lectins and Lipopolysacharides as linking Agents for the Red Cell Linked Antigen Test

Immunology 1970, 18, 353-360

TDI has been used in this work as a coupling agent. Lectins and lipopolysacharides were treated with TDI and then added to various antigens to produce coupled reagents which were capable of reacting with the surface of red cells. The red cells "relabelled" in this way functions as a reagent capable of detecting antibodies.

The coupling procedure with TDI in aqueous medium seems very doubtful . This is not an investigation on the effect of TDI on sera or living beings.

2. Interaction between Isocyanates and Enzymes

2.1 W.E. Brown, F. Wold: Alkyl Isocyanates as Active Site-Specific Inhibitors of Chymotrypsin and Elastase

Science 174, No. 4009, 608-10, 1971

The reaction of octyl isocyanate (OIC) and butyl isocyanate (BIC) with the pancreatic enzymes chymotrypsin and elastase in a buffer (pH 7,6) is studied at room temperature. OIC and BIC were added in acetone solution to the aqueous protein solution; the molar ratio of the isocyanates to the enzymes was varied between 50 : 1 and 1 : 1. The reaction occurred rather quickly and was complete on all isocyanate concentrations in less than 30 sec whereas the half life time of their hydrolysis reaction was about 1 minute. This confirms that the reaction with the protein has preference over the water reaction. The enzyme derivates were isolated and contained 1 mol isocyanate to 1 mol enzyme.

Both chymotrypsin and elastase were inactivated by BIC; OIC inactivates only chymotrypsin. The authors discuss a two step reaction between the protein and the isocyanate, namely the formation of a non-covalent complex to be followed by covalent binding in case the aliphatic chain of the isocyanate fits in the "binding pocket" of the protein. It is confirmed that other pancreatic enzymes like trypsin and carboxypeptidase are not affected even in case of an 100 fold molar excess of OIC.

The possibility that isocyanates may represent unique chemical "yard sticks" to determine the dimension of a protein molecule is discussed.

2.2 J. Twu, F. Wold: Butyl Isocyanate, an Active-Site-Specific Reagent for Yeast Alcohol Dehydrogenase

Biochemistry, vol. 12, no. 3, 381-386, 1973

L-cysteine and butylisocyanate (BIC) form a stable adduct identified as S-(butylcarbamoyl)-L-cysteine (BCC). This compound is quite stable within the pH 2-6 range. In the pH 10 range complete decomposition occurs at 4°C/19 h incubation as well as at 30°C/1 h incubation. Cystin appeared to be the product of decomposition.

BIC is also combined with yeast alcohol dehydrogenase. The disappearance of SH-groups during the reaction indicates that these groups are the sites for the modification of the protein. The resulting peptide is isolated and characterized. S-(butylcarbamoyl)-cysteine is formed by enzymatic digestion of the peptide. The reaction is performed in aqueous buffered medium at pH <6,5-7,0. The conclusion is drawn that the inactivation of the enzyme is caused by reaction of BIC with specific sulfhydryl groups in "three of the enzyme's four active sites". The enzyme can be protected against BIC-inactivation by specific coenzymes. It is claimed that each active site contains 2 SH-groups one of wich only reacts with the BIC.

2.3 W.E. Brown, F. Wold: Alkyl Isocyanates as Active-Site-Specific Reagents for Serine Proteases. Identification of the Active-Site Serine as the Site of Reaction

Biochemistry, vol. 12, no. 5, 835-840, 1973

The adducts of radioactive (C_{14}) butylisocyanate (BIC) on chymotrypsin and elastase have been totally hydrolysed under the influence of proteolytic enzymes. In both cases O-(C_{14}-butylcarbamoyl)-serine has been identified as the amino acid derivative to which most of the BIC is linked. The BIC adduct to chymotrypsin has been subjected to performic acid oxidation and digestion with trypsin and other enzymes. Three peptides have been isolated finally which represent 21 % of the radioactivity at the beginning. It was shown that the active site serine-195 was derivatised by BIC.

By a similar treatment of the (C^{14})butylcarbamoyl elastase peptide containing products were found accounting for 63 % of the basic activity. This larger peptide contains four serine residues but only one of these - the active site serine-188 - has obviously been derivatised in the reaction of elastase with BIC.

2.4 I. Twu, C.C.Q. Chin, F. Wold:

Studies on the Active Site Sulfhydryl Groups of Yeast Alcohol Dehydrogenase

Biochemistry 12, 2856-2862 (1973)

It has been claimed that yeast alcohol dehydrogenase contains two distinct sulfhydryl groups on each active site but that only one of which reacts with butyl isocyanate.

The characteristics of the reactions of these sulfhydryl groups with butyl isocyanate and with iodoacetamide and some properties of the inactive derivates have been studied further. Conditions of experiments were similar as described on 2.2.

Except for the reaction with iodoacetamide at pH 6,5 the two sulfhydryl groups per active site cannot both be modified; when one has reacted, the other becomes unreactive towards either reagent. It is concluded that two sulfhydryl groups are closely associated.

2.5 W.E. Brown, F. Wold:

Alkyl Isocyanates as Active-Site-Specific Reagents for Serine Proteases. Reaction Properties

Biochemistry, vol. 12, no. 5, 828-834, 1973

The main objective of the investigation is to ascertain the specifity of reaction of serine proteases - chymotrypsin, elastase, trypsin - with octyl isocyanate (OIC) and butyl isocyanate (BIC). Chymotrypsin is inactivated by both isocyanates; elastase reacts with BCI, but not with OCI; trypsin is not affected by either reagent. The selectivity in the reaction of the three proteases with OCI and BCI can be explained on the basis of their "binding pockets" which were indicated by X-ray diffraction analysis.

The inactivation of an enzyme requires an intact "unprotected site" of the protein. E.g. an inactive form of chymotrypsin produced by protonation or by treatment with indol does not react with isocyanates.

The kinetics of hydrolysis of the two aliphatic isocyanates are assessed, their analytical determination during the reaction with isocyanates is described. It is confirmed that OIC and BIC react much faster with the enzymes than with water.

2.6 P.D. Snyder jr., F. Wold, R.W. Bernlohr, C. Dullum, R.J. Desnick, W. Krivit, R.M. Condie:

Enzyme Therapy II, Purified Human α-Galactosidase A - Stabilization to Heat and Protease Degradation by Complexing with Antibody and by Chemical Modifiation

Biochimica et Biophysica Acta 350, 432-436, 1974

Purified human α-galactosidase A was treated with hexamethylene diisocyanate (HDI) in acetone solution. The reaction took place in a pH 6.5 phosphate buffer. HDI caused a cross-linking of the enzyme resulting in a markedly increased thermal stability compared to the native enzyme. It was also shown that the resistance to trypsin and protease digestion was remarkable compared to the non treated enzyme. Inactivation of this enzyme occurred when treated with butyl isocyanate.

No information is available on the mechanism of the isocyanate enzyme reaction.

2.7 R.M. Krupka:

On the Anti-cholinesterase Activity of Benomyl

Pestic. Sci. 1974, 5, 211-216

The fungicide Benomyl is a butylcarbamate of a benzimidazol-derivative. It is known for some time that this compound is a cholinesterase inhibitor. The author has performed a series of experiments which confirm that the inhibition effect is caused by butylisocyanate which is a breakdown product of Benomyl. It is formed from Benomyl in presence of water under the conditions of the test which is used to evaluate the cholinesterase inhibiton potency of a compound. The data from the experiments suggest that butylisocyanate reacts "in statu nascendi" rather quickly with the cholinesterase before it is hydrolysed to butylamine.

The other breakdown product (benzimidazol-derivate) does not show any inhibition effect on the enzyme.

2.8 W.E. Brown:

Alkyl Isocyanates as Active Site-Specific Reagents for Serine Proteases. Location of Alkyl Binding Site in Chymotrypsin by X-Ray Diffraction

Biochemistry, vol. 14, no. 23, 5079-5084, 1975

The structure of octylcarbamoyl-α-chymotrypsin is evaluated by α-ray diffraction. The n-octyl chain of the isocyanate (OIC) binds specifically in the binding pocket of the substrate. The isocyanate group forms an urethane bond with the OH-group of the serine 195. The finding that the active site of the enzyme "accomodates" OIC which according to theoretical considerations and data from earlier studies has to be regarded as a too large molecule is discussed thoroughly. The stability of the OIC link to the enzyme is a valuable contribution to the assessment of the "topography" of the active site in the chymotrypsin molecule. It is confirmed that the urethane link of OIC to the serine hydroxyl is stable to all normal protein and peptide isolation techniques.

2.9 M. Gross, N.K. Whetzel, J.E. Folk:

Alkyl Isocanates as Active Site-directed Inactivators of Guinea Pig Liver Transglutaminase

Journal of Biological Chemistry, vol. 250, no. 19, 7693-7699, 1975

Several aliphatic isocyanates (methyl-, n-butyl-, n-hexyl-, ethyl- and n-propyl-isocyanate) were found to act as effective inactivators of guinea pig liver transglutaminase in the presence of Ca-ions at pH from 5.7 - 7.5. Only Isopropyl-isocyanate failed to inactivate the enzyme effectively.

The hypothesis that the inactivation results from the formation of an alkyl-thiocarbamate ester from the single active site sulfhydryl-group of the enzyme is supported by the loss of the only free -SH group and complete loss of activity by the incorporation of one mol of the isocyanate per mol of the enzyme. The chemical properties of the adducts are quite similar to those reported earlier for an other alkyl-thiocarbamoyl enzyme and an alkyl-thiocarbamoyl cysteine derivative.

Ureas formed form the reaction of the aliphatic isocyanates with water did not show any inactivation effect. It is confirmed that the rate of the -SH/-NCO reaction is much faster than the -NCO/water reaction.

2.10 M. Manno, M. Lotti: Cholinesterases in Human Toluendiisocyanate Exposure

Int. Arch. Occup. Environ. Hlth. 38, 55-50 (1976)

The authors studied the serum and red cell cholinesterase activities on 30 workers of a toluene diisocyanate (TDI) manufacturing plant. The examination of the respiratory function was carried out in parallel. A significant decrease of red cell cholinesterase activity compared to a control group was found in 70 % of the cases. The inhibition of serum cholinesterase activity was not significant. Also no significant bronchoconstriction phenomena were found.

2.11 W. Ardelt, A. Koj, J. Chudzik, A. Dubin: Inactivation of Some Pancreatic and Leucocyte Elastases by Peptide Chloromethyl Ketones and Alkyl Isocyanates

FEBS Letters, vol. 67, no. 2, 156-160, 1976 (North-Holland Publishing Company, Amsterdam)

The inactivation of some pancreatic and leucocyte elastases by the addition of butyl- and octyl-isocyanate was studied. The "incubation" of the enzymes with the isocyanates was performed at 30°C for 5 minutes in a pH 7.4 phosphate buffer using solution of the isocyanates in acetone The concentration of the isocyanates at a given enzyme concentration was determined which was required to reduce the activity of the enzyme by 50 %. It is shown that at an enzyme concentration of 1-3 µM approx. 3.10^{-2} µM of butyl-isocyanate was sufficient to cause this effect. Up to 40 times the amount of octyl-isocyanat compared to butyl-isocyanate was needed to inhibit the enzyme to the same degree.

No details of the chemical aspects of the enzyme/isocyanate reaction are presented.

2.12 H. Ogawara:

Penicillin Isocyanates for β-Lactamase

Methods in Enzymology, vol. XLVI, Affinity Labeling, Academic Press, London, 1977

Benzylpenicillin isocyanate and isocyanate derivates of other penicillins are obtained via the Curtius-reaction. The effect of these isocyanates as well as butyl- and phenylisocyanate on the activity of β-lactamase is studied. The enzymatic activity is more strongly reduced by the isocyanates in an acidic condition than in a neutral or alkaline condition. The findings suggest that the isocyanates react first with the histidine residue in its protonated form followed by a reaction with a neighboring (amino?) group to form a stable and inactivated protein.

Hydrolysis of the β-lactam ring under the influence of β-lactamase of the benzylpenicillin isocyanate occurs simultaneously to the inactivation of the enzyme. The rate of hydrolysis is 100 times less than with benzylpenicillin.

The paper contains no data on the reaction of the respective isocyanates with water.

2.13 F. Ogata, K. Ninomiya, N. Yoshida, S. Makisumi:

Chemical Modification of Trypsin with Alkyl Isocyanates

Memoirs of the Faculty of Science, Kyushu University Ser. C, 10 (1978) 205-214

Octyl isocyanate is an active site directed inactivator of chymotrypsin. It has been supposed that the hydrophobic pockets of the enzyme first bind the n-alkyl side chain of the isocyanate to form a noncovalent intermediate, analogous to an enzyme-substrate complex. The specific binding is followed by a covalent linkage of the isocyanate group to a serine in the active site of the enzyme.

In the present work the effect of methylguanidine to the carbamylation of trypsin with short chain isocyanates was investigated. Contrary to expectation the presence of methylguanidin and benzamidine gave a significant protection of the active serine groups against inactivation reaction with propylisocyanate. Ethyl and butyl isocyanates reacted analogously. The isocyanate modified trypsin preparations still exhibit catalytic activity.

2.14 R. McKenna, T. Ahmad, H. Frischer:

Correlation Between Glutathione Reductase Activity and ADP Induced Platelet Aggregation

Clin. Res. 26, No. 5, 669 A, 1978

Chloroethylisocyanate is a potent inhibitor of glutathione reductase whereas methyl-, ethyl- and n-butyl-isocyanate were less powerful inhibitors of this enzyme. These compounds produced a significant blockade of adenosin-diphosphate platelet aggregation. There is no indication for an inhibition of lipoamide dehydrogenase by chloroethylisocyanate.

2.15 T. Ahmad, H. Frischer:

Inactivation of Glutathione Reductase and of Lipoamide Dehydrogenase by BCNU and Alkyl-isocyanates

Clin. Res. 26, No. 5, 701 A, 1978

Human red cell glutathione reductase is profoundly inhibited by alkylisocyanates with linear side chains containing 8 or fewer C-atoms. Bulkier or longer compounds are inactive. Yeast glutathione reductase and pig heart lipoamide dehydrogenase are markedly inhibited by n-propyl- or n-butylisocyanate.

2.16 R. McKenna, T. Ahmad, H. Frischer:

Does Platelet Function Depend on Glutathione Reductase (GSSG-R) Activity?

Thromb. Haemostasis 42, No. 1,12, 1979

2-Chloroethylisocyanate and chloromethyl-isocyanate were shown to be potent inhibitors of human red cell glutathione reductase and abolished the aggregation of platelets induced by adenosin-diphosphate. There is an indication that the platelet dysfunction under the influence of these isocyanates is related to deficiency of glutathione reductase.

2.17 A. Trevisan, G. Moro: Role of Acetylcholinesterase Inhibition in Toluene Diisocyanate (TDI) Induced Bronchoconstriction

Int. Arch. Occup. Environ. Health 49, 129-135, 1981

In vitro studies by incubating TDI with acetylcholinesterase (AChE) in acetone solution confirm that TDI causes an inhibition of the AChE which is significant only for high TDI concentrations. On in vivo studies 13 subjects (devided in 3 groups) were exposed to TDI around the 0,02 ppm level for 30 minutes. In addition to controls of the respiratory function a possible influence of TDI on the AChE activity was studied on blood serum samples of the subjects. An average inhibition of the AChE 4 h after the exposure of 6,13 and 16 % was found on the 3 groups. The paper does not discuss in detail the biochemical aspect of the TDI/AChE "reaction". It is excluded that TDI undergoes a link to the NH_2-groups of the AChE's esterase site whereas a biochemical effect mediated by an immune mechanism is suggested. The authors feel that AChE plays an essential role in the development of TDI bronchoreactivity on individuals with a clinical history of bronchial asthma.

18 W.E. Brown, A.H. Green, M.H. Karol, Y.C.E. Alarie: Inhibition of Cholinesterase Activity by Isocyanates, 1982

(Submitted for publication)

Hexamethylendiisocyanate (HDI), hexylisocyanate (HI) and 2,6 toluene diisocyanate (2,6 TDI) were found to inhibit completely human serum cholinesterase at molar ratios of 4:1 to 8:1 (isocyanate:enzyme). In contrast molar ratios of 50:1 or greater were required for 50 % enzyme inhibition by 2,4 TDI, phenylisocyanate (PhI) or o-Tolyl-isocyanate (o-TI). If purified cholinesterases were exposed to atmospheres containing 1 ppm isocyanates also inhibition of the enzyme was observed. HDI and HI were the most potent inhibitors whereas 2,4 TDI and 2,6 TDI showed much less reactivity. HDI and HI were still potent enzyme inhibitors when whole human plasma was used as the source of cholinesterase.

The isocyanates were added to the enzymes as solutions in acetone.

The authors take into account that isocyanates as used in the study might undergo side reactions in the aqueous medium in which all trials were made. These side reactions, however, were not quantified; their influence if any on the cholinesterasis inhibition was not studied.

3. Antigens based on adducts of isocyanates and proteins

3.1 L. D. Scheel, R. Killens, A. Josephson:

Immunochemical Aspects of TDI Toxicity

Am. J. Ind. Hyg. 25 (1964) 179-184

The method for synthesis of antigens composed of crystalline egg albumin conjugated to TDI is described. From the reaction of the antigens with the inhalation exposure antibodies it is concluded that TDI when inhaled reacts with constituents of the body to produce an antigen which in turn leads to antibody formation bearing TDI specifity.
TDI injected intravenously into rabbits and rats causes a fever response.

3.2 R.B. Konzen, B.F. Craft, L.D. Scheel, C.H. Gorski:

Human Response to Low Concentrations of p.o-Diphenylmethane Diisocyanate (MDI)

Am.Ind.Hyg.Assoc.J. 25, 121-127 (1966)

The immunological test used by the authors indicates that an exposure of humans to MDI aerosol of 1.3 ppm · min resulted in an antibody response, whereas an exposure of about 0.9 ppm · min did not. This study indicates that detection of MDI antibodies in the serum of individuals is diagnostic proof of a recent exposure to isocyanates.

Six months after exposure to MDI no antibodies could be detected.

3.3 H. E. Stokinger, J. T. Mountain, L. D. Scheel:

Pharmacogenetics in the detection of the hypersusceptible worker

Ann. N. Y. Acad. Sci. 151 (1968) p. 968-76

The article, among other work, describes a predictive test for hypersensitivity to isocyanates prior to exposure. Isocyanate antigens made from TDI or MDI reacted with serum globulin according to Scheel et al or polygen, an allergen composed of several pollen extracts are used in the Prausnitz-Kustner test for passive cutaneous anaphylaxis according to Layton. There is indication that those allergic to the polygen may also become hypersensitive to the isocyanates.

.4 H.C.Bruckner, S.B.Avery, D.M.Stetson, V.N.Dodson

Clinical and Immunologic Appraisal of Workers Exposed to Diisocyanates

Arch.Environ.Health 16(1968)619-625

Forty-four isocyanate-exposed and unexposed workers of Wyandotte Chemicals Corp. were examined clinically, their work environment investigated, and their blood studied for evidence of an immunologic response to TDI. Repetitive inhalation exposures to excessive concentrations is important in isocyanate sensitization. Observations suggest that an allergy diathesis seems to predispose one to developing hypersensitivity to isocyanate compounds. Immunologic response to isocyanates was detected best by the lymphocyte transformation test. Results were negative with all other tests, probably because isocyanate exposure dated back more than six months. A TDI-HSA conjugate was used as the test antigen in all of these tests.

3.5 S.B.Avery, D.M.Stetson, P.M.Pan, K.P.Mathews

Immunological Investigation of Individuals with TDI Asthma

Clin.exp.Immunol. 4(1969)585-596

This investigation is a continuation of the work of H.C.Bruckner et al. (Arch.Environ.Health 16 (1968)619.
In an attempt to document an immunological response to TDI in the blood of workers who appear clinically to be sensitized, several conjugates of TDI with human serum albumin were prepared.
Most of the tests failed to show antibodies to TDI. In lymphocyte culture, however, TDI-human serum albumin complexes produced stimulation of lymphocytes from seven of the eight subjects suspected of being sensitized and none of the controls.
In the preparation of TDI-HSA the authors were aware of the possible side-reactions; therefore the procedure of Scheel, Killens and Josephson(1964) was modified: TDI in dioxane was added slowly to 1% aqueous HSA solution at p_H 4,5. Concentration of TDI in dioxane was varied so that approximately equal volumes of TDI-dioxane solution and albumin solution were mixed.
The possible unsuitability of the TDI-HSA test antigens is discussed.

Further the authors point out, that the occurence of some forms of immunological response to drugs and other low molecular weight compounds is not necessarily associated with clinical manifestations of hypersensitivity. So this work is considered only to support, but not to prove the hypothesis that TDI asthma is allergic.

3.6 G. Taylor:

Immune Responses to TDI Exposure in Man

Proc.Roy.Soc.Med. 63, 379-380 (1970)

It has been demonstrated that exposure to TDI vapour may give rise to circulating antibodies in man. Three methods are presented to detect antibodies in the serum of humans exposed to TDI:

1. Complement fixation method (CFT):

   Bovine serum albumin (BSA) was conjugated with 2,4-TDI. Unconjugated BSA is used as control antigen. The serum is tested with 3 antigen concentrations.

2. Red cell linked antiglobulin technique (RCLAT):

   TDI is combined with sheep erythrocytes in a pH 7.4 buffer. The "sensitized" cells were incubated with the serum and combined with a rabbit antihuman globulin reagent.

3. Passive cutaneous anaphylaxis test (PCA):

   The serum is injected intradermally into the abdominal skin of cynomologues monkeys. The animals after intravenous administration of Evans blue sufficient to cause "bluing" of the gums were exposed to saturated TDI vapour for 3 minutes. Areas of bluing were red after 30 minutes.

It is suggested that these three techniques differ either in specifity or immunoglobulin class. The etiological relationship between circulating antibody and symptoms of clinical sensitivity remains to be investigated. Although approximately half the subjects tested gave positive results, as yet the techniques available do not provide a suitable laboratory diagnostic test for TDI sensitivity. None of the 40 control sera gave positive results.

3.7 C.V.Porter, R.L.Higgins, L.D.Scheel

A retrospective Study of Clinical, Physiologic and Immunologic Changes in Workers Exposed to TDI

Am.J.Ind.Hyg.Assoc. 36, 159-168 (1975)

A retrospective study was made of workers in a TDI plant(Allied Chemical Corp.) using an immunologic test procedure developed at the NIOSH laboratories. Case histories of 32 workers in seven response groupings are presented. Sensitization was found to correlate with the frequency and severity of significant exposures to TDI. The immunologic change which produces tolerance in most workers exposed to TDI results in a state of "immunologic anergy" which gives a negative response when TDI antigen challenge tests are performed. This correlation with the clinical observations in exposed workers is highly significant in this study. Some individuals exposed to TDI may have protective antibodies but still experience bronchoconstriction.

-3.8 M.H.Karol, H.H.Ioset, E.J.Riley, Y.C.Alarie

Hapten-specific Respiratory Hypersensitivity in Guinea Pigs

Am.Ind.Hyg.Assoc.J. 39(1978)546-556

Respiratory hypersensitivity to small chemical determinants (haptens) was produced in guinea pigs by repeatedly exposing the animals to aerosols of hapten-ovalbumin conjugates. In this way, reactivity toward p-tolyl-isocyanate was induced. Hapten-specific respiratory hypersensitivity was accompanied by the production of hapten-specific antibodies. The method for inducing hapten-specific hypersensitivity can be applied to screen various industrial chemicals for their sensitizing abilities toward the respiratory tract.

9 M.H.Karol, H.H.Ioset, Y.C.Alarie

Tolyl-specific IgE Antibodies in Workers with Hypersensitivity to TDI

Am.Ind.Hyg.Assoc.J. 39(1978)454-458

Incorporation of a p-tolyl(mono)-isocyanate-human serum albumin (TMI-HSA) antigen conjugate into a solid phase radioimmunoassay permitted detection of tolyl-specific IgE antibodies in 3 of 4 workers with clinical hypersensitivity to TDI. By comparison, 19 TDI-exposed, non-sensitized workers had antibody titers similar to those found in normal adults. High titers of tolyl-specific IgE antibodies were not correlated with high levels of total serum IgE. Use of the monofunctional isocyanate in place of TDI in antigen preparation prevented crosslinking of antigen protein, an effect usually associated with TDI, and also assured that tolyl groups were sterically exposed. TMI-HSA antigens may prove beneficial in serological or cutaneous evaluation of TDI-sensitized workers.

3.10 M. H. Karol, Y. C. Alarie:

Tolyl isocyanate test antigens, methods for their preparation and use in detecting diisocyanates and antibodies to diisocyanates

US 4 208 399 (16.8.78)
≙ GB 2 028 339
≙ GE-OS 2 921 761

Appl.: University of Pittsburgh

A test antigen for TDI-antibodies was prepared by reacting p-tolylisocyanate with buffered 1 percent aqueous solution of human serum albumin (HSA). The conjugate was dialized and spectroscopically characterized. There were 10 moles of hapten bound to 1 mol HSA.

Sera of either TDI-sensitized, TDI-exposed but non-sensitized and non-exposed individuals were investigated by paper radioimmunosorbent test (PRIST).

The group of TDI-exposed , non-sensitized workers had antibody titers (measured as net counds per minute, cpm) comparable low to those found in sera from blood bank donors. The TDI-sensitized group, on the other hand, displayed markedly elevated titers of antitolyl antibody with the exception of an induvidual unexposed to TDI for at least two years prior to this study.

Claim 1: An antigen for detection of a selected diisocyanate comprising the reaction product of a protein and an monoisocyanate derivate of said selected diisocyanate.

3.11 C. S. T. Tse, S. E. Chen, T. L. Bernstein:

Induction of Murine Antibodies by TDI
An Animal Model of Immediate Hypersensitivity Reactions to Isocyanates

Am. Rev. of Respiratory Disease 120 (1979) 829-835

The purpose of this investigation was to develop a reproducible murine model of homocytotropic antibody (HCA) induced by chemically stable isocyanate-protein conjugates. Formation of TDI-specific HCA was observed in a susceptible mouse strain and was studied further as a possible animal model analogous to immediate hypersensitivity-like reactions in workers exposed to TDI.

Hapten-protein conjugates (antigens) have been prepared by reaction of TDI, o-tolyl- and p-tolyl-isocyanate, with 1 percent phosphate-buffered solutions of human serum albumin (HSA), bovine serum albumin (BSA) and ovalbumin (OVA). Purification of the conjugates has been done by centrifuging and dialyzing, thus removing unreacted TDI as well as low molecular reaction products. In the purified aqueous solutions the number of moles to isocyanate bound to protein as a function of reaction time has been determined spectrophotometrically.

Mice were immunized with these conjugates. Sera were assessed for passive cutaneous anaphylaxis. Hapten specifity was proved by absorption experiments. Anaphylactic activity was complete ly abolished after heating at 56° for 1 hour.

Inhibition of HCA activity by both mono- and diisocyanate protein conjugates not only confirms that these antibodies are hapten specific, but also suggest that the tolyl moiety may be an important haptenic determinant. Both homologous and heterologous o-tolyl adducts show uniform inhibitory effects. Preparation and characterization of the protein-isocyanate conjugates seem to be questionable (short time heterogenous reaction).

3.12 M.H.Karol, T.Sandberg, E.J.Riley, Y.Alarie

Longitudinal Study of Tolyl-Reactive IgE Antibodies in Workers Hypersensitive to TDI

J.Occup.Med. 21 (1979) 354-358

Three workers with TDI hypersensitivity were evaluated for IgE antibodies to TDI over a period of 13 months. A radioallergosorbent test (RAST) system was employed using p-tolyl-isocyanate-human serum albumin antigen.
IgE antibody titers were consistently elevated in two individuals who experienced several bronchial hypersensitivity responses to TDI during the study period.
By contrast, antibody titers in a third subject who had not experienced any hypersensitivity reactions during the study period continually decreased, falling to insignificant levels after 12 months. In the absence of renewed TDI exposure, sensitive workers may have titers indistinguishable from those of workers exposed to TDI but without sensitivity to the chemical.
It is apparent that the tolyl-specific radioimmunoassay will identify TDI-sensitized persons only if they have been exposed to TDI within several months prior to serologic evaluation.

3.13 M.H.Karol, E.J.Riley, Y.Alarie

Presence of Tolyl-Specific IgE and Absence of IgG Antibodies in Workers Exposed to TDI

J.Environ.Sci.Health, C 13(3),221-232 (1979)

Test antigens used to evaluate human sera for antibodies to TDI were prepared by reaction of p-tolyl(mono)-isocyanate with either ovalbumin or human serum albumin. The latter antigen had 10 moles of tolyl hapten per mole HSA. The molecular sizes of various TDI and tolylisocyanate conjugates were estimated by gel electrophoresis. As it had been expected (Am.Ind.Hyg.Assoc.J.39(1978)546), electrophoresis of TDI-conjugates indicated only a small portion of the reaction products remained sufficiently unpolymerized to permit migration into the SDS gel. The multiple bands observed in these samples indicated formation of numerous polymeric species.
Independent immunoenzymatic and radioimmunoassay techniques indicated the absence of tolyl-specific IgG antibodies in sera from 25 workers with long-term exposure to TDI. Five of these workers were clinically hypersensitive to TDI and displayed significant titers of tolyl-specific IgE antibodies in their sera.

3.14 C.S. Ted Tse, A.J. Pesce:

Chemical Characterization of Isocyanate-Protein Conjugates

Toxicology and Appl. Pharmacol. 51, 39-46 (1979)

The rates of reaction of TDI and HDI with human serum albumin (HSA) were studied. The reaction was performed in a phosphate buffered saline at pH 7.4.

TDI reacts preferably with NH_2-groups of the protein (presumably of the lysin) forming ureid linkages but the formation of urethane linkages cannot be excluded. Most of the TDI reacted bifunctionally.

In the case of HDI addition mainly occurred on the NH_2-groups of the lysin component of the HSA but it became evident that adducts were formed also with other reactive groups of the protein. Experimental conditions and analytical procedures seem to be questionable. The chemical structures proposed are similar to those of classical hapten derivatives and suggest that such derivatives may be immunogenic and / or allergenic in some workers exposed to the vapors of those diisocyanates.

3.15 I.M.O'Brien, M.G.Harries, P.S.Burge, J.Pepys

TDI-Induced Asthma
I.Reactions to TDI,MDI,HDI and Histamine

Clinical Allergy 9(1979)1-6

Twenty-four workers handling diisocyanates and with respiratory disease were investigated by occupational-type bronchial provocation tests for sensitivity to TDI, to which all were exposed, to MDI and HDI. Sixteen gave asthmatic reactions to TDI and eight of these also reacted to MDI. Four of the eight TDI and MDI reactors had histories of exposure only to TDI, and of them two reacted also to HDI. Of nine subjects tested with HDI, three gave asthmatic reactions, and all three also reacted to TDI and MDI.

3.16 I.M.O'Brien, A.J.Newman-Taylor, P.S.Burge, M.G.Harries I.W.Fawcett, J.Pepys

TDI-Induced Asthma
II.Inhalation challenge tests and bronchial reactivity studies

Clinical Allergy 9(1979)7-15

In sixty-three workers exposed to TDI, no overall differences in bronchial reactivity to histamine inhalation and to exercise testing were found between the total groups of positive and negative TDI reactors to provocation tests.
TDI highly sensitive subjects were also highly sensitive to histamine. The findings suggest that, on the one hand, the asthmatic reactions to TDI cannot be attributed solely to non-specific mechanisms and, on the other, that in subjects with high degrees of specific sensitivity non-specific mechanisms may also be playing a part.

3.17 M.H.Karol, C.Dixon, M.Brady, Y.Alarie

Immunologic Sensitization and Pulmonary Hypersensitivity by Repeated Inhalation of Aromatic Isocyanates

Toxicology and Appl.Pharmacology 53 (1980) 260-270

The present study reports successful sensitization of animals by inhalation of p-tolyl isocyanate (TMI) or TDI on 5 consecutive days. Within 2 weeks antibodies specific for tolyl (mono- or di-)isocyanate were detected. Antibodies to TMI reacted with TMI-protein conjugates but did not react with conjugates containing TDI, n-hexyl isocyanate, or phenyl isocyanate. By contrast, antibodies to TDI cross-reacted with TMI-protein and phenyl isocyanate-protein antigens, but did not cross-react with conjugate antigens which contained hexyl isocyanate as the haptenic group. Utilization of the animal model to establish dose-response and threshold concentration relationships may provide a means to establish safe exposure levels for industrial workers exposed to reactive chemicals which may act as haptens.
The following conjugate antigens were prepared: p-tolyl isocyanate-guinea pig serum albumin (TMI-GSA), p-tolyl isocyanate-bacterial amylase (TMI-BA), phenyl isocyanate-guinea pig serum albumin (P-GSA) and TDI-GSA. Each of the GSA conjugate antigens contained 20 to 30 mol of isocyanate hapten per mole protein on the average. TMI-BA had an average of five TMI groups/mol BA.

3.18 X.Baur, W.Dorsch, G.Fruhmann, H.Römmelt, P.Roth, W.Diller

Klinische Symptomatik und Ergebnisse von RAST und inhalativem Provokationstest

Zentralbl.Arbeitsmed. 30(1980)104-109

This investigation refers to the relevance of the radio-allergo-sorbens test (RAST) which has been developped further, for the clinical diagnosis of asthma. Correlation of concentration of isocyanate specific IgE antibodies with inhalative threshold concentration, duration and intensity of TDI exposition is examined. Contrary to earlier interpretations the results indicate that the pathogenic reactions caused by TDI are in part based on a specific hypersensitivity mediated by IgE antibodies. An allergy diathesis of the respiratory tract seems to predispose hypersensitivity to TDI. On the other hand no relationship could be found between such a TDI-hypersensitivity and an allergy diathesis of the skin.

3.19 M.H.Karol, Y.Alarie

Antigens which Detect IgE Antibodies in Workers Sensitive to TDI

Clinical Allergy 10(1980)101-109

Two antigens, both containing tolyl groups, were compared for ability to detect IgE antibodies in workers hypersensitive to TDI. One antigen, formed by reaction of p-tolyl isocyanate with human serum albumin, detected antibodies in each of ten hypersensitive workers. A second tolyl antigen, formed by reaction of p-toluoyl chloride and human serum albumin, and therefore lacking isocyanate linkages, detected antibodies in seven of the ten workers. Hexyl-ureido-HSA (prepared by reaction of n-hexyl isocyanate with HSA detected antibodies in guinea pigs sensitized to hexyl isocyanate, but did not react with antibodies produced in animals sensitized to TDI or with antibodies from workers with TDI hypersensitivity.

3.20 M. H. Karol:

Study of Guinea Pig and Human Antibodies to Toluene Diisocyanate

Am. Rev. respiratory disease 122 (1980) 365-70

TDI asthma has long been believed to have an IgE antibody-mediated pathogenesis. Several investigators were successful in detecting TDI-specific antibodies in sensitized persons, but others were not. The different results have been ascribed to the problem of preparing satisfactory hapten-protein conjugates because of the bifunctionality of TDI, which may cause cross-linking and side reactions.

In order to avoid this problem and to ensure sterically exposed hapten groups antigens werde prepared using o-, m-, and p-tolylisocyanate.

This study compared the three tolyl monoisocyanate isomers with TDI for haptenic ability to detect antibodies to TDI in sensitized guinea pigs and human workers.
Result showed that tolyl-specific IgE antibodies were not present in sera from nonsensitized guinea pigs and nonsensitive workers. After exposure to TDI sera of the guinea pigs reacted differently in degree with all haptens prepared. Antibodies in TDI-sensitized workers , as determined by radiallergosorbent testing (RAST), reacted comparably with each of the haptenes.

3.21 C. R. Zeiss, T. M. Kanellakes:
J. D. Bellone, D. Levitz,
J. J. Pruzansky, R. Patterson:

Immunoglobulin E-mediated asthma and hypersensitivity pneumonitis with precipitating anti-hapten antibodies due to diphenylmethane diisocyanate exposure

J. Allergy Clin. Immunol. 65 (1980), 346-352

Two workers who were exposed for a long time to MDI developed quite divergent clinical syndromes of hypersensitivity.
The antibody response of these workers to a conjugate of MDI with human serum albumin (MDI-HSA) was measured by various methods. Both workers had serum IgE antibody specific for MDI-HSA, but precipitating antibody was found only in the serum of one of them.
Results indicate that a marked immunologic response to MDI is possible in exposed workers and that hypersensitivity pneumonitis can occur subsequent to the inhalation of MDI.

4. Pharmacological effects of isocyanates

4.1 J.R. Tittensor, M.D. Edge, G.J. Stacey, R. Howe:

Nucleosid-derivates

GE-OS 2412 981 (March 18, 1974)
Priority GB (March 21, 1973)
Appl. Imperial Chemical Industries Ltd., London

Modification of cyclic adenosin-monophosphate (cAMP) by adding an aliphatic or aromatic monoisocyanate (e.g. butylisocyanate, chlorophenyl-isocyanate, phenylisocyanate) to the NH_2-group in the adenin part of the cAMP.

The new substances are considered as improved pharmaceuticals to inhibit the aggregation of platelets.

4.2 M. van Ert, M. C. Battigelli:

Mechanism of Respiratory Injury by TDI

Ann. Allergy 35 (1975) 142-47

Since there is no unequivocal evidence of antibodies caused by TDI exposure and having in mind that TDI is a primary irritant, the authors look for a direct pharmacological effect of TDI.

Even the hypersensitivity phenomenon could be explained on the basis of a beta-adrenergic dysfunction predating the TDI exposure, which the exposure itself would further aggravate.

The pharmacological effect of TDI was investigated in a set of in vitro experiments using peripheral leukocytes from human blood as model tissue.

Results:
TDI is not a histamine releaser per se, but may contribute to the action of histamine and possibly of other related mediators by moderating the β-adrenergic function, a recognized histamine antagonist.
TDI reduces the CAMP stimulating effect by catecholamines. In this regard, TDI behaves as a blocker of beta-adrenergic function.
TDI inhibits glucagon stimulation of CAMP and causes unequivocal limitation of antigenic release of histamine.

4.3 B.T.Butcher, J.E.Salvaggio, C.E.O'Neil, H.Weill, O.Garg

Toluene Diisocyanate Pulmonary Disease: Immunopharmacologic and Mecholyl Challenge Studies

J.Allergy Clin.Immunol. 59(1977) 223-227

Selected workers exhibiting clinical sensitivity to TDI were studied for: (1) in vitro TDI-induced leukocyte histamine release; (2) determination of cyclic 3'5'adenosine monophosphate (cAMP) levels of lymphocytes exposed to TDI; (3) effect of TDI on the isoproterenol-induced increase of lymphocyte cAMP levels; and (4) acetyl-ß-methylcholine (mecholyl) inhalation challenge. TDI did not induce histamine release from leukocytes of sensitive or nonsensitive individuals, nor were lymphocyte cAMP levels affected by in vitro TDI exposure. TDI did, however, diminish in vitro stimulation of cAMP by isoproterenol. Mecholyl challenge suggest that TDI-induced obstructive airways disorders may be associated with altered ß-adrenergic function.

4.4 B. T. Butcher, R. M. Karr, C. E. O'Neil, R. J. Davies, J. E. Salvaggio:

TDI pulmonary disease: studies of mediators and inhalation challenge testing in sensitized workers.

J. Allergy Clin. Immunol. 61 (1978) 138

Short communication on in-vivo investigation with sensitive and non sensitive TDI workers.
Cyclic adenosin monophosphate (CAMP stimulation of lymphocytes by TDI inhalation was measured. Results suggest a quantitative or qualitative difference of cell adrenergic receptors for sensitive and nonsensitive individuals. There may be a class of workers who respond only to high levels of TDI.

4.5 G.K. Sangha, Y. Alarie: Sensory Irritation by Toluene Diisocyanate in Single and Repeated Exposures

Toxicology and Applied Pharmacology 50, 533-547, 1979

The authors describe effects of single and repeated exposures to TDI at various concentrations on mice. The influence of TDI on the respiratory rate of the animals has been followed in body plethysmographs for periods of up to 240 min on each individual exposure. Exposures were repeated up to three times on consecutive days.

The finding that the rate of recovery of animals was dependent on the duration of exposure is interpreted as a cumulative effect. The authors believe that a pharmacological mechanism could explain their observation. They feel that TDI reacts with the protein of the nerve endings in the respiratory tract. They suggest that in the first phase a noncovalent complex is formed to be followed by the formation of a covalent adduct - characterised by an urethane, urea or other link. The rapid recovery after short exposures is related to the formation of the complex whereas the slow recovery upon longer exposure would be due to the formation of the adduct.

No in vitro experiments have been made to support this theory.

4.6 R.J.Davies, B.T.Butcher, C.E.O'Neil, J.E.Salvaggio

The in vitro Effect of TDI on cyclic adenosine monophosphate (cAMP) production by isoproterenol(ISO), prostaglandin (PGE) and histamine. A possible mode of action.

J.Allergy Clin.Immunol. 60(1979) 223-229

Since no correlation had been found between the presence of specific serum IgE antibody against a TDI-HSA conjugate and proved TDI-induced asthma, the authors investigated pharmacologic mechanisms by which TDI might induce asthma in man. The nature and site of the reaction by which TDI stimulates lymphocyte AMP in vitro remains speculative. TDI alone significantly increases intracellular cAMP levels in vitro. It produces a four- to fivefold increase in lymphocyte cAMP at a concentration of 10^{-3} M, a TDI concentration which appears to have no inhibitory effect on cAMP production by either ISO or PGE. Conversely, at the lower concentration of $3,3 \cdot 10^{-4}$ M, TDI does not significantly increase intracellular cAMP but significantly reduces cAMP stimulation by ISO and PGE. This evidence argues against a direct interaction between TDI and ISO or PGE in the reaction mixture and could probably be best explained in terms of a partial agonist activity.
TDI has no significant effect on the production of lymphocyte cAMP following incubation with histamine. The problem of reacting a TDI-solution in DMSO with an aqueous medium is discussed.

5. Nitrosoureas - Supposed pharmacological mechanism of antitumor activity

5.1 B.B. Baril, E.F. Baril, J. Laszlo:

Effect of 1,3-Bis(2-Chloroethyl)-1-Nitrosourea (BCNU) on Purified Rat Liver DNA Polymerases

Clin. Res. 21, no. 3, 643 (1973)

1,3 bis(2-chloroethyl)-1-nitrosourea (BCNU) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) inhibit DNA synthesis of intact and disrupted leukemic cells; the DNA template is not primarily affected.

The authors show that only purified DNA polymerase II is inhibited by BCNU and CCNU. DNA polymerase I which they have also isolated from the rat liver is not inhibited by the two substances. The corresponding isocyanates - 2-chloroethyl-isocyanate and cyclohexyl-isocyanate - which following the authors are formed from the respective ureas BCNU and CCNU (??) were found to be more active inhibitors than the ureas. DNA polymerase I was also not inhibited by these isocyanates.

The authors state that BCNU and CCNU as well as the isocyanates are known to react with the ε-amino group of lysin. It is assumed that a reaction with protein serine is also possible.

5.2 H.E. Kann, K.W. Kohn, J.M. Lyles:

Inhibition of DNA Repair by the 1,3-Bis (2-chloroethyl)-1-nitrosourea Breakdown Product, 2-Chloroethyl Isocyanate

Cancer Research 34, 398-402 (1974)

A marked inhibition of repair of single strand DNA breaks has been produced by 2-chloroethyl isocyanate (CIC). It is claimed but not proven that CIC is formed as breakdown product of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) which is known to act as antitumor agent. The repair was not inhibited by 2-chloroethylamine. It is tentatively suggested that the cytocidal effect of BCNU may be potentiated by one of its "metabolites" namely CIC through an inhibition of the repair of damaged DNA.

5.3 B.B. Baril, E.F. Baril, J. Laszlo, G.P. Wheeler:

Inhibition of Rat Liver DNA Polymerase by Nitrosoureas and Isocyanates

Cancer Research 35, 1-5 (1975)

1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) and 1-(2-chloroethyl)-3-(trans-4-methyl-cyclohexyl)-1-nitrosourea (MeCCNU) are effective antitumor agents when tested against various animal neoplasias. By a treatment of DNA-polymerase I with these compounds and the isocyanates corresponding to BCNU - 2-chloroethyl-isocyanate, CIC - and to CCNU - cyclohexyl-isocyanate, CYCI - no alteration of the activity of this enzyme was found whereas 30 - 45 % inhibition of the enzymatic activity of DNA polymerase II by BCNU and CCNU was found. CIC and CYCI caused an inhibition of 75 and 90 %. The amines corresponding to these isocyanates are not influencing the activity of DNA polymerase II.

The authors assume but have not proven that isocyanates will be formed from the ureas (?!) and that they are responsible for the inactivation of this enzyme.

5.4 J. Hilton, F. Maldarelli, S. Sargent:

Evaluation of the Role of Isocyanates in the Action of Therapeutic Nitrosoureas

Biochemical Pharmacology 27, 1359-1363 (1977)

The half-lives of chloroethyl (CIC)- and cyclohexyl-isocyanate (CYCI) in tissue culture medium have been determined. The figure obtained for CYCI was 10 times higher than for CIC (~17 sec.). Basing on these figures and on the data for the half-live times for 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) the isocyanate concentration produced during the breakdown of BCNU and CCNU has been calculated. L 1210 or Hela cells exposed either to the nitrosoureas or to equivalent concentrations of the respective isocyanates showed no deficiency in the repair of gamma irradiation damage. It is stated that isocyanates play a minor role in the overall cytotoxicity of nitrosoureas.

5.5 J.R. Babson, D.J. Reed, M.A. Sinkey:

Active Site Specific Inactivation of Chymotrypsin by Cyclohexyl Isocyanate Formed during Degradation of the Carcinostatic 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea

Biochemistry 16, no. 8, 1584-1589 (1977)

The authors found that an incubation of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) with chymotrypsin resulted in covalent modification and inactivation of this enzyme. Because cyclohexyl-isocyanate was shown to be an active site inhibitor of chymotrypsin it is assumed that the inactivation is due to the formation of cyclohexyl-isocyanate from the nitrosourea compound. No experimental work has been done to confirm this assumption. (From the chemical point of view the formation of an isocyanate from an urea particularly in an aqueous environment has to be regarded as extremely improbable).

5.6 A.J. Tornace, K.W. Kohn, H.E. Kann:

Inhibition of the Ligase Step of Excision Repair by 2-Chloroethyl Isocyanate, a Decomposition Product of 1,3-Bis(2-chloroethyl)-1-nitrosourea

Cancer Research 38, 1064-69 (1978)

The authors present a scheme following which 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) is decomposed in a biological environment into chloroethyl-isocyanate (CIC) and a chloroethyl-carbonium ion. They claim that the ligase step of excision repair on damaged DNA strands is inhibited by CIC. In fact CIC is applied as solution in absolute ethanol and will consequently react immediately with this solvent to form the respective ethylurethane. This compound may therefore be the inhibitor and not the isocyanate.

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